TESTBIOTECH Background 25 - 08 - 2022

Testbiotech comment on EFSA's assessment of genetically engineered oilseed rape MON 94100 for food and feed uses, under regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2020-169) from Bayer

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Introduction
The EFSA GMO panel assessed the oilseed rape MON 94100 which was developed to confer tolerance to dicamba herbicide. Oilseed rape MON 94100 expresses two variants of the DMO protein: DMO and DMO + 27. The DMO protein demethylates dicamba, producing 3,6-dichlorosalicylic acid and formaldehyde, conferring tolerance to dicamba-based herbicides. The inserted gene construct involves genetic information from two viruses, two plants (from other species) and from bacteria.

1. Systematic literature review

A systematic literature review as referred to in Regulation (EU) No 503/2013 was provided by the applicant. However it seems, the review was narrowly focused on the specific event. It did not identify any peer-reviewed publications. The applicant should have applied much broader research also taking into account other transgenic plants which were made resistant to dicamba. Furthermore, at least in regard to environmental risks, it is necessary to also review literature which might indicate indirect, delayed and cumulative long term risks, also on interaction with other genetically engineered plants which might occur from spillage and further crossings. Therefore, the literature research should especially take into account potential persistence, spread and crossings with other transgenic plants which also enter the environment via spillage along transport routes etc. In this context, also the biological characteristics of potential offspring are relevant for the application. Therefore, literature research should include all relevant publications concerning the crop species and its relatives. Furthermore, also the environmental risk assessment should take into account indirect, unintended, delayed and long-term cumulative effects of animal excretions. Therefore, literature research should include all genetically engineered plants which may become mixed in the diet and may cause environmental hazards.

In addition, in regard to food and feed safety, there is the need to consider interactions with other genetically engineered plants which might be mixed with the event in diets. Implementing Regulation 503/2013 (point 3.2.3) requests that "*the applicant shall evaluate the data generated to estimate possible short-term and long-term risks to human or animal health associated with the consumption of genetically modified food or feed with respect to the expression of new proteins/metabolites, as well as significantly altered levels of original plant proteins/metabolites.*" Apparently, this legal request is not limited to the specific event. It comprises risk assessment of mixed diets in equivalence to risk assessment of stacked events, since also the risks are equivalent. Therefore, also a much more comprehensive literature review is needed about potential interactions with other regulated GMOs.

2. Molecular characterisation and gene expression

Annex II of Implementing Regulation 503/2013 requests that

- "*Protein expression data, including the raw data, obtained from field trials and related to the conditions in which the crop is grown (in regard to the newly expressed proteins).*" (Scientific requirements 1.2.2.3)
- "*In the case of herbicide tolerant genetically modified plants and in order to assess whether the expected agricultural practices influence the expression of the studied endpoints, three test materials shall be compared: the genetically modified plant exposed to the intended herbicide; the conventional counterpart treated with conventional herbicide management regimes; and the genetically modified plant treated with the same conventional herbicide management regimes."* (Scientific requirements 1.3.1)
- "*The different sites selected for the field trials shall reflect the different meteorological and agronomic conditions under which the crop is to be grown; the choice shall be explicitly justified. The choice of non-genetically modified reference varieties shall be appropriate for the chosen sites and shall be justified explicitly.*" (Scientific requirements 1.3.2.1)

Open reading frames and gene insertion

The genetic engineering process led to the emergence of many new open reading frames in the genome of the plants. In order to assess the sequences encoding the newly expressed proteins, or any other open reading frames (ORFs) present within the insert and spanning the junction sites, it was assumed that proteins that may emerge from these DNA sequences would raise no safety concerns. Other gene products, such as ncRNAs (non-coding RNA) from additional open reading frames, were not assessed. This is astonishing since not only the boarder sequences may give raise to biological active molecules. Also the complex gene construct as inserted in the genome, should be considered as a potential source of biological active molecules which are not present in the conventionally bred comparators. The inserted construct comprises genetic information from two viruses, two other plants species and bacteria: The expression cassette which was introduced consists of the PClSV promoter from peanut chlorotic streak caulimovirus, the 5' untranslated leader sequence from the RNA of tobacco etch virus (TEV), a sequence encoding 27 amino acids of the chloroplast targeting peptide from the Rubisco (rbcS) gene of *Pisum sativum*, and from an intervening sequence (DMO + 27), the coding sequence of the dicamba mono-oxygenase gene (dmo) from *Stenotrophomonas maltophilia*, and the 3' untranslated sequence of the guf-Mt1 gene from *Medicago truncatula*.

In addition, two further (combined) expression cassettes were used for transformation (with further transgenes from plants, viruses and bacteria), which are supposed to be segregated by further breeding. However, no full whole genome sequencing was applied to detect unintended remaining fragments or other unintended genetic alterations which are unlikely to occur from conventional breeding. Instead the genome was only screened for specific sites.

Thus, uncertainties remain about other biologically effects arising from the method of genetic engineering and the newly introduced gene constructs (for potential uptake of the molecules for example see also Davalos et al., 2019). In this case, 'Omics' should be applied to investigate the rate of intended and unintended gene products under various genetic conditions. Without this information, the next steps in risk and exposure assessment, can not be conducted. It should not be overlooked that Regulation 503/2013 under point 1.4.2 also requests "*testing of new constituents other than proteins*".

Impact of environmental factors, agricultural practice and genetic backgrounds
The data presented by Bayer do not meet the requirements of Implementing Regulation 503/2013: (1) the field trials were not conducted in all relevant regions where the GE plants may be cultivated, and extreme weather conditions were not taken into account systematically; (2) the field trials did not take all relevant agricultural management practices into account; (3) no sufficiently broad range of relevant genetic backgrounds was taken into account.

Data on environmental factors, stress conditions and their impact on gene expression
Data was only presented from field trials carried out at five sites in the US and Canada for just one year. No unusual weather conditions are mentioned in the EFSA opinion. It seems that in some regions, drought conditions were observed, however irrigation was applied. In summary, EFSA concludes "*that the meteorological data set falls within the historical range of climatic conditions normally occurring at these sites.*" This might be true. However, it is not sufficient to fulfill the legal provisions which request data which are representative for all "*different meteorological and agronomic conditions under which the crop is to be grown"*. In this regard, the regions chosen and the climatic conditions described do not seem to be sufficient. In addition, it is wrong to only consider the historical data, since the plants are about to be grown in future under the conditions of ongoing climate change. Therefore, the plants should also be exposed to experimental climatic conditions (for example in climate chamber) to make sure, the necessary data on gene expression are made available.

However, no experiments were requested to show to which extent specific environmental conditions may influence the gene expression of the additionally inserted genes. Hence, data as made available seem not to be sufficient to fulfill the requirements of Implementing Regulation 503/2013 to assess whether the expected environmental conditions under which the plants are likely to be cultivated will influence the expression of the studied endpoints.

Data on herbicide application rates and their impact on gene expression
It is quite unusual that the applicant can not give empirical data on the expected range of herbicide applications but only some reasoned estimation. Under these conditions, the highest dosage should be applied that can be tolerated by the plants and which may be applied if herbicide resistant weeds are occurring in the fields. Also repeated spraying should be applied, since this may become agronomic practice.

In light of the information available, we assume that the application and the data provided may not sufficiently represent the agricultural practices, which could include the use of each of the herbicides alone and higher dosages.

Consequently, the GE plants tested in field trials are likely to not sufficiently represent the products intended for import. The data presented by the applicant are insufficient to conclude on the impact of the herbicide applications on gene expression, plant composition or biological characteristics of the plant as requested in EU Regulation 503/2013.

Impact of genetic backgrounds on gene expression
It is known that the genomic background of the varieties can influence both the expression of the inserted genes and plant metabolism (see, for example, Lohn et al., 2020; Trtikova et al., 2015). Therefore, EFSA should also have requested additional data from transgenic plant varieties.

However, EFSA has not taken these issues into consideration. Consequently, the GE plants tested in field trials are likely to not sufficiently represent the products intended for import. The data

presented by the applicant are insufficient to conclude on the impact of the genetic backgrounds on gene expression as requested in EU Regulation 503/2013.

3. Comparative assessment of plant composition and agronomic and phenotypic characteristics

Implementing Regulation 503/2013 requests:
"*In the case of herbicide tolerant genetically modified plants and in order to assess whether the expected agricultural practices influence the expression of the studied endpoints, three test materials shall be compared: the genetically modified plant exposed to the intended herbicide; the conventional counterpart treated with conventional herbicide management regimes; and the genetically modified plant treated with the same conventional herbicide management regimes.*"

"*The different sites selected for the field trials shall reflect the different meteorological and agronomic conditions under which the crop is to be grown; the choice shall be explicitly justified. The choice of non-genetically modified reference varieties shall be appropriate for the chosen sites and shall be justified explicitly.*"

The data presented by Bayer do not meet the requirements of Implementing Regulation 503/2013: (1) the field trials were not conducted in all relevant regions where the GE plants will be cultivated, and no sufficiently defined extreme weather conditions were taken into account; (2) the field trials did not take all relevant agricultural management practices into account; (3) no sufficiently broad range of relevant genetic backgrounds (belonging to several maturity groups) was taken into account.

Data on environmental factors and stress conditions - and their impact on plant composition and phenotype

Field trials to assess plant composition as well as agronomic and phenotypic characteristics of the GE plants were only conducted in the US and Canada at eight sites and just for one year. No unusual weather conditions are mentioned in the EFSA. It seems in some regions, drought conditions were observed, however irrigation was applied. In summary, EFSA concludes "*that the meteorological data set falls within the historical range of climatic conditions normally occurring at these sites.*" This might be true. However, it is not sufficient to fulfill the legal provisions which request data which are representative for all "*different meteorological and agronomic conditions under which the crop is to be grown"*. In this regard, the regions chosen and the climatic conditions described do not seem to be sufficient. In addition, it is wrong to only consider the historical data, since the plants are about to be grown in future under the conditions of ongoing climate change. Therefore, the plants should also be grown under experimental and controlled climate conditions (for example in climate chamber) to make sure, the necessary data on gene expression are made available.

However, no experiments were requested to show to which extent specific environmental conditions influence plant composition and agronomic characteristics. Hence, data as made available seem not to be sufficient to fulfill the requirements of Implementing regulation 503/2013 to assess whether the expected environmental conditions under which the plants are likely to be cultivated will influence the expression of the studied endpoints.

Data on herbicide application rates and their impact on plant composition as well as agronomic and phenotypic characteristics

It is quite unusual that the applicant can not give empirical data on the expected range of herbicide

applications but only some reasoned estimation. Under these conditions, the highest dosage should be applied that can tolerated by the plants and which may be applied if herbicide resistant weeds are occurring in the fields. Also repeated spraying should be applied, as this may become agronomic practice.

In light of the information available, we assume that the application and the data provided do not sufficiently represent the agricultural practices, which could include the use of the each of the herbicide alone and higher dosages.

EFSA should have requested the applicant to submit data from field trials that include many more agricultural practices, active ingredients, dosages and all combinations of the herbicides that might be used in agricultural practice of the GE plants producing countries. Without these data, no reliable conclusion can be drawn as requested in Implementing Regulation 503/2013 (in particular for herbicide tolerant GE plants) to assess whether anticipated agricultural practices influence the outcome of the studied endpoints (see also Miyazaki et al., 2019).

Consequently, the GE plants tested in field trials are likely to not sufficiently represent the products intended for import. The data presented by the applicant are insufficient to conclude on the impact of the herbicide applications on gene expression, plant composition or biological characteristics of the plant as requested in EU Regulation 503/2013.

Impact of genetic backgrounds on plant composition as well as on agronomic and phenotypic characteristics

It is known that the genomic background of the varieties can influence both the expression of the inserted genes and plant metabolism (see, for example, Lohn et al., 2020; Trtikova et al., 2015). Therefore, EFSA should also have requested additional data from transgenic plant varieties.

However, EFSA has not taken these issues into consideration. Consequently, the GE plants tested in field trials do not sufficiently represent the products intended for import. The data presented by the applicant are therefore insufficient to conclude on the impact of the genetic backgrounds on gene expression as requested in EU Regulation 503/2013.

Data from compositional and phenotypical analysis show the need for further investigations

For agronomic and phenotypic analysis, only ten criteria were considered. Three of those criteria were significantly different to the data from comparator if not treated with the complementary herbicide, four after treatment. Three of the endpoints were consistently found, with and without spraying. This underlines that the process of genetic engineering is a likely cause of these changes.

In regard to compositional analysis, 13 out of 46 criteria were significantly different to the data from comparator, no matter if or if not treated with the complementary herbicide. Some of these findings fell into the highest category of significant differences. The same endpoints were consistently found being different, with and without spraying. This underlines that the process of genetic engineering is a likely cause of these changes.

Given the above reasoning on the impact of environmental factors, herbicide applications and genetic backgrounds, EFSA should have requested more data: data on agronomic and phenotypic endpoints should be generated from a wider range of clearly defined stress factors, including all relevant agricultural practices and genetic backgrounds. In addition it is not acceptable that only data from kernels were used for compositional analysis. Since the whole plants may enter the environment via spillage, also data from the whole plant are necessary for their risk assessment.

In any case, more data and a more detailed analysis would have been necessary to investigate changes in plant composition and phenotype, and also to investigate potential unintended changes in metabolic pathways and the emergence of unintended biologically active gene products.

The material derived from the plants should have been assessed by using omics techniques to investigate changes in the activity of the transgene and the plant genome, and also to investigate changes in metabolic pathways and the emergence of unintended biologically active gene products. Such in-depth investigations should not depend on findings indicating potential adverse effects, they should always be necessary to draw sufficiently robust conclusions to inform the next steps in risk assessment.

In addition, in awareness of the absence of any independent data on this GE plants (see literature review, EFSA, 2022a), we strongly recommend establishing a system with independent controls to repeat the trials and double check the data on plant composition and agronomic characteristics.

Conclusion on the comparative assessment of plant composition as well as on phenotypic and agronomic characteristics

Based on the available data, no final conclusions can be drawn on the safety of the plants. Therefore, the data neither fulfill the requirements of Implementing Regulation 503/2013 nor Regulation 1829/2003. This is also underlined by several statements made by experts from Member States (EFSA, 2022b).

In summary, the GE plants tested in the field trials do not sufficiently represent the products intended for import.

4. Toxicity

- Implementing Regulation 503/2013 requests:
- "*Toxicological assessment shall be performed in order to:*
- *(a) demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health;*
- *(b) demonstrate that unintended effect(s) of the genetic modification(s) identified or assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health;*"
- "*In accordance with the requirements of Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that:*
- *(a) the genetically modified food and feed has no adverse effects on human and animal health;*"

Effects of residues from spraying with complementary herbicide specific to GE plants and their mixed toxicity

The residues from spraying were considered to be outside the remit of the GMO Panel. However, without detailed assessment of these residues, no conclusion can be drawn on the safety of the imported products: due to specific agricultural management practices in the cultivation of the herbicide-resistant plants, there are, for example, specific patterns of spraying, exposure, occurrence of specific metabolites that require special attention. It should not be overlooked that, without

genetic engineering, dicamba was not used as an on top herbicide in oilseed rape. This specific application only occurs in genetically engineered plants and therefore, its hazards and risks have to be assessed before approval of food & feed products derived.

Both, EU pesticide regulation and GMO regulation, require a high level of protection for health and the environment. Thus, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered a prerequisite for granting authorisation.

EU legal provisions such as Regulation 1829/2003 (and Implementing Regulation 503/2013) state that "*any risks which they present for human and animal health and, as the case may be, for the environment*" have to be avoided.

Therefore, potential adverse effects resulting from exposure to whole food and feed need to be tested for mixed toxicity (EFSA, 2019b). This need to assess the whole food and feed should also be considered in regard to changes in the intestinal microbiome. For example, Liao et al., 2021 describe effects of dicamba on soil organisms, causing prevalence of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in soil microbiomes. Similar or different effects may also be relevant for the intestinal microbiome at the stage of consumption and therefore needs to be taken into account in case of dicamba resistant GE plants. The described effects which may enhance the uptake of DNA from the transgenic plants by gut bacteria, are not considered under pesticide regulation, they have to be assessed within GMO risk assessment. The reason: These effects are highly dependent on the specific dosages applied on the GE plants, their metabolism and the resulting pattern of exposure in food and feed. Also cumulative effects (mixtures of GE plants in one diet) may play a decisive role. Under Directive 2001/18/EC such effects could be considered as indirect effects which may be immediate, delayed or cumulative. Implementing Regulation 503/2013 (point 1.4.2) requires "*testing of new constituents other than proteins*". To our opinion this requirement includes also the assessment of residues of the complementary herbicides which necessarily become constituents of all genetically engineered plants which were made resistant to it.

In regard to food and feed safety, EFSA (2020) considers microbiomes to be highly relevant to the health status of their hosts. Therefore, it is desirable to understand the importance of their role in risk assessment. EFSA expects that gut microbiome research (not only in the case of GE plants) will play a relevant role in regulatory science with potential implications for future risk assessments and predictive risk models. As EFSA states: "*considering that the gut microbiome is a biological component directly and indirectly involved in the metabolism of food/feed components and chemicals and in the protection of the host against adverse environmental exposure, it would be useful to establish criteria on how to evaluate the potential adverse impacts of perturbators on this defensive barrier, and consequently, on human/animal health.*"

In general, antibiotic effects and other adverse health effects might occur from exposure to a diet containing these plants that were not assessed under pesticide regulation. These adverse effects on health might be triggered by the residues from spraying with the complementary herbicide. Further attention should be paid to the specific toxicity of the metabolites of the pesticide active ingredients that might occur specifically in the GE plant and therefore might escape pesticide regulation.

However, no attempts have been made to integrate the microbiome into the risk assessment of food and feed derived from the GE plants. This is in direct contradiction to Regulation 1829/2003 which requests "*genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken*

under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment." (Recital 9).

Feeding study

A 90 day feeding study was performed, however only with low dosages (15% and 5%). The safety of rapeseed oil has not been documented by this study, carried out in the absence of a lipid fraction, present in too small an amount in delipidated rapeseed oil cake. This is a major deficiency since oil will be the most relevant product when it comes to exposure of consumers via the food chain.

Several significant effects were observed as also summarized in the comments from several Experts of Member States. These findings also include dose-related changes and specific data such as

- lower mean haemoglobin, haematocrit and also red blood cells. The mean haemoglobin level is reported to be outside the range of the historical control data which, according to the experts from member states, may indicate that the administration of GM oilseed rape meal has adversely affected blood haematology in the test group animals.
- Statistically significantly higher mean testes weight was measured in the "test group high" male animals. The mean for this group is reported to be outside the range of the historical control data.
- Statistically significantly higher mean heart weight relative to final body weight.
- Increased organ weights (testes in males, relative uterus weight in females, as well as pituitary, adrenal glands, and relative weights of adrenal gland, heart and liver in combined sex).

However, EFSA did not see the need for further, more targeted investigations. One reasoning is that no histopathology findings were reported. This is a relevant argument. However the findings from the feeding study might also indicate physiological reactions under chronical exposure effects in histopathological findings.

It should not be overlooked that Implementing Regulation 503/2013 (point 3.2.3) requires that "*the applicant shall evaluate the data generated to estimate possible short-term and long-term risks to human or animal health associated with the consumption of genetically modified food or feed with respect to the expression of new proteins/metabolites, as well as significantly altered levels of original plant proteins/metabolites.*" We come to the conclusion that this requirement, which for example also comprises long term accumulated effects, is not fulfilled and safety was not demonstrated.

Environmental risk assessment

Oilseed rape (*Brassica napus*) can spread via pollen and seeds, and seeds can remain viable in the soil for more than ten years (seed dormancy). Europe is the centre of origin and genetic diversity for the group of *Brassica* plants to which oilseed rape belongs. Some native plant populations, such as *Brassica rapa* (turnip), can hybridise with oilseed rape. *Brassica napus* itself occurs mainly as a cultivated plant, but still maintains significant characteristics of a wild plant. Disturbed soil promotes the establishment of *Brassica napus* beyond the fields, whereas dense vegetation will hinder establishment. However, *Brassica napus* growing in the wild is found primarily in habitats where wild relatives of the *Brassica* genus and related genera grow. In addition, many related species which can hybridise with oilseed rape occur in environments such as road verges, industrial or feral sites. Gene flow to wild relatives is possible and likely to happen, even if *Brassica napus* itself only has a reduced potential to spread in a densely vegetated environment (Bauer-Panskus et al., 2013). A recent publication (Sohn et al., 2021) shows that the uncontrolled spread of genetically

engineered (GE) oilseed rape is already happening in 14 countries on five continents. These are countries which either allow the cultivation of GE oilseed rape (such as the USA and Canada), or have tested it in experimental releases (such as Germany), or allow the import of kernels (such as Japan). Moreover, it has to be assumed that there is a high number of undetected cases, as many regions do not have systematic monitoring. In many cases, the plants have persisted in or around the fields and along of transport routes for several years, and have been found to have a higher potential for environmental spread than previously assumed.

Also in case of MON94100 it is likely that the plants will persist in the environment after spillage and start to propagate. This would allow next generation effects to emerge that were neither assessed by the applicant nor by EFSA (Bauer-Panskus et al., 2020). They also may cross with other GE oilseed rape which already entered the environment via spillage before. However, risk assessment of EFSA did not assess direct and indirect effects which maybe immediate, delayed or accumulated as required by Directive 2001/18/EC in the case of environmental risk assessment. Instead EFSA claims that no further uncertainties were detected, which indicates an extraordinary degree of intended ignorance. In any case, the EU Commission should not allow the import of viable kernels.

Others

If approval for import is given, the applicant has to ensure that post-market monitoring (PMM) is developed to collect reliable information on the detection of indications showing whether any (adverse) effects on health may be related to GM food or feed consumption. Thus, the monitoring report should at very least contain detailed information on: i) actual volumes of the GE products imported into the EU, ii) the ports and silos where shipments of the GE products were unloaded, iii) the processing plants where the GE products was transferred to, iv) the amount of the GE products used on farms for feed, and v) transport routes of the GE products. Environmental monitoring should be run in regions where viable material of the GE products such as kernels are transported, stored, packaged, processed or used for food/feed. In case of losses and spread of viable material (such as kernels) all receiving environments need to be monitored. Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing GE products during or after the production process, and during or after human or animal consumption should be part of the monitoring procedure (see also comments from Member States experts, EFSA, 2022b).

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